Eco. Env. & Cons. 27 (October Suppl. Issue) : 2021; pp. (S183-S193)
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ISSN 0971–765X

Comparative Expression Studies of thirteen transcripts in *Tectona grandis* L.f. under different irrigation treatment using qRT-PCR

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(Received 18 December, 2020; accepted 2 February, 2021)

ABSTRACT

In recent climate change phenomenon, drought is the major environmental factor which badly affects the forest leading to its mortality. *Tectona grandis* L.f. (Teak) is a tropical forest tree species famous for its high durability wood quality. Woody plant with its deep root system withstand drought to some extent. However, seedling establishment is very much needed for genetic improvement and breeding program in this harsh environmental condition. Understanding the plant physiology and molecular mechanism under drought is very helpful for developing high variety genetic stock. Being a major Teak producing country, there are scanty of report on gene expression pattern of teak under drought. Understanding up regulated and down regulated transcripts and their expression under drought will help us to find more details about their expression and exploring more data on it. We report here the change in the expression pattern of 13 Teak transcripts in 25 selected samples of 3 drought tolerant cultivars in different irrigation schedule of 100%, 75%, 50% and 25%. qPCR studies were performed on two chemistries, Taqman and Sybr Green Based. Taqman based studies were performed for the transcript TR19630 | c0_g1, TR26753 | c0_g1, TR52087 | c0_g4, TR26739 | c0_g3, TR16494 | c0_g4, TR54668 | c0_g1, TR41913 | c0_g1, 18S rRNA and Sybr Green based studies were performed for the transcripts TR83729 | c0_g2, TR20915 | c1_g1, TR15761 | c0_g1, TR38214 | c0_g1, TR41764 | c0_g1 TR15734 | c0_g2. When the tree is subjected to 25% drought condition, TR19630 | c0_g1, TR41764 | c0_g1, TR26753 | c0_g1, TR16494 | c0_g4, TR15734 | c0_g2, and TR20915 | c1_g1 showed increased expression. The expression of transcripts TR54668 | c0_g1, TR26753 | c0_g1, TR26739 | c0_g3, TR19630 | c0_g1 and TR41913 | c0_g1 has been significantly high just by inducing 100% irrigation treatment. TR38214 | c0_g1 has shown lower expression in all the drought condition. Further bioinformatics bases pathway analysis can clearly elucidate the mechanism by which these transcripts exert their function on teak tree leaves leading to apoptosis.

Key words: *Tectona grandis, Drought stress, Gene expression, Forest, India*

Introduction

Drought stress is the major environmental factor which badly affects the whole forest ecosystem leading to decline of forest productivity and thus mortality. Drought mainly affects the plant by changing its morphological and physiological characteristics and biochemical and the molecular mechanism. Though forest trees or other crop plant have drought avoidance mechanism to some extent, plants are unable to

survive in extreme environmental condition. Drought tremendously effects the seedling establishment which is the main cause of forest depletion. In recent days it's a serious issues and it is very important to understand the plants molecular mechanism to avoid drought so that it will help in genetic improvement and breeding program. Drought stress mainly effect the physiological parameters like photosynthesis, transpiration, stomatal conductance, water use efficiency, root shoot ratio, plant biomass etc. Increased proline and chlorophyll content are also indicators of drought stress. Proline, as an osmolyte helps plant to maintain homeostasis under drought stress. Thus understanding all the physiological parameters and then further study of molecular mechanism under drought stress will help us to select the germplasm which in turn will help on reforestation program. The negative consequences of drought became even more urgent in current times of climate change because projections suggest that such events will occur more frequently and become extreme (Allen *et al*., 2010; Reyer *et al*., 2015)

Teak (*Tectona grandis* L. f) is an economically important woody plant species under the family Lamiaceae which is known for its high durability wood quality. Though this species grow naturally in wide environmental condition, the optimum conditions are in areas having rain fall between 1200 and 2500 mm (Kaosa-ard, 1981, Tripathi *et al.*, 2017). Teak is mainly native to Myanmar, Indonesia, Srilanka, Laos etc. (Lyngdoh *et al*., 2010; Palupi *et al*., 2010). In India, Teak grows mainly in Andhra Pradesh, Karnataka, Kerala, Maharashtra, Tamilnadu, Uttar Pradesh. In North eastern region also, teak plantations are found. Moist, warm and tropical climate is mainly suitable for Teak. In recent days, its popularity leads to its own threat. Therefore it will be very helpful if superior germplasm of Teak species which is drought tolerance can be screened and included in the forest management system. Deep tap root system of teak can withstand drought but proper watering is necessary for seedling establishment.

There are several reports on Teak physiology and growth. However, most of our knowledge on drought responses of plants at the molecular level is based on laboratory experimental conditions of dehydration and/or osmotic treatments (Abe *et al.*, 1997; Shinozaki and Yamaguchi-Shinozaki 1997; Oono *et al.*, 2003; Umezawa *et al*., 2004). Knowing the molecular mechanism behind drought is utmost important to all of us. Trancriptomic study of model plant like Arabidopsis helps us to go through the details of the gene expression pattern under drought (Zhang *et al*., 2004). Due to the large genome size in trees, sometimes it is very challenging for the researchers to sequence the whole genome. Thus to reduce the complexity of the analysis, and minimizing the sequencing cost, candidate genes offer a valuable tool in expression studies. Metabolic process, transport processes are the important pathways affected during limited water supply. Different types of Heat shock protein (TR50354|c0_g1) and HSP20-like chaperones super family protein, stress enhanced protein 1 (TR10362|c0_g1) and osmotin CDPK9, auxin (AUX/ IAA transcriptional regulator family protein), ethylene responsive elements are reported unregulated in drought tolerant Teak plant (Tripathi *et al.,* 2017). Previously reported Teak trancriptomics study (Tripathi *et al*., 2017) helps us to find several unique transcripts under drought. Understanding those transcripts and their expression will help us to find more details about their expression under drought and exploring more data on it. Being a major Teak producing country, there are scanty of report on gene expression pattern of teak under drought. Our aim was to have a comparative expression of transcripts in Teak from different selected cultivars based on physiological parameters under irrigation treatment of 25%, 50%, 75%, 100% of field capacity.

Materials and Methods

Plant materials and stress treatment

Tectona grandis L.f. accessions of parent plants were collected from different region of North East India. The plants were chosen randomly from different population. The collected accessions were grown in CSIR North East Institute of Science & Technology, Jorhat, Assam, India experimental farm nursery. The six month old accessions were planted in polybag with a mixture of sand soil. Sand soil was mainly used for its low water holding capacity. The accessions were then transferred to plastic shade house and arranged in factorial experiment in complete randomized design with three replicates represented by nine plants. Field capacity was calculated according to Klute (1986) and the irrigation treatments were applied once in an interval of 7 days using normal tap water. Irrigation schedule was

maintained at 100%, 75%, 50%, 25% of field capacity. The plants were kept at dehydration stress up to 6 month. Out of 41 accessions, 3 drought tolerant accessions were selected on the basis of vegetative and physiochemical properties in which percentage of survival recorded was 100 while the rest failed to survive. The selected accessions were taken for the gene expression study. The samples selected for the expression profiling were given in Table 1.

Table 1. Samples selected for expression study

Sl. No.	Cultivers	Sample Id	Treatment
1	Control	1	Control
2	GKU24	3	25%
3	GKU24	4	25%
4	CHM37	9	25%
5	CHM37	11	25%
6	BNU10	13	25%
7	BNU10	14	25%
8	GKU24	6	50%
9	GKU24	7	50%
10	CHM37	8	50%
11	CHM37	16	50%
12	BNU10	21	50%
13	BNU10	15	50%
14	GKU24	18	75%
15	GKU24	10	75%
16	CHM37	12	75%
17	CHM37	17	75%
18	BNU10	20	75%
19	BNU10	22	75%
20	GKU24	24	75%
21	GKU24	5	100%
22	GKU24	23	100%
23	CHM37	25	100%
24	CHM37	2	100%
25	BNU10	19	100%

RNA extraction and quantification

mRNA was extracted from Leaf samples of Teak Tree by Trizole-LS reagent (Invitrogen, USA) according to the manufacturer's protocol. Isolated RNA was then quantified by Nanodrop 2000 (Thermo Scientific), and the 260/230 ratio was approximately 2.0 for most of the samples. The intregrity of RNA was also checked by running Agarose gel electrophoresis.

cDNA conversion and quantification

mRNA product obtained was then reverse transcribed to cDNA by using cDNA synthesis kit - SuperScript™ IV First-Strand Synthesis System (Cat No# 18091050) from Invitrogen, Thermo Fisher Scientific, with the following reaction conditions with denaturation at 94 °C for 2 min, annealing at 55 °C for 30 sec for a cycle and final extension at 68 °C for 1 min for 35 cycle.

Gene expression analysis by Real Time PCR

The Gene expression studies were performed to decipher the change in the expression pattern of 13 Teak transcripts (Table 2) in 25 selected samples of 3 drought tolerant cultivars in different irrigation schedule of 100%, 75%, 50%, and 25%. The selected transcripts were taken from the previously published transcriptomic study result of Teak (Tripathi *et al.,* 2017). Quantitative real time PCR was conducted to compare the expression of selected transcripts in the treated samples and also in the control untreated sample which was grown in natural environment. qPCR was performed with Quant Studio 3 Real Time PCR from Applied Biosystems-Thermo Fisher Scientific. qPCR studies were performed on two chemistries, Taqman and SYBR Green Based. For Taqman Based Studies, 7 Taqman Assays were custom designed based on the primer sequences provided in Table 2. Taqman Based reaction was set up using 20 µl reaction volume by adding reaction mix 10 µl 2X Taqman Mastermix,1 µl 20 X ERBB2 PP mix and 9µl of cDNA.

For Sybr Green Based studies, 6 primers were taken (Table 2). A total reaction volume of 20 µl Sybr Master Mix were prepared by mixing 10 µl 2X Power Up SYBR Green Master Mix, 1 µl Forward Primer, 1 µl Reverse Primer and 8 µl cDNA. The total cycle conditions for both Taqman and Sybr Green based studies were 95 °C melting for 15 sec, 62 °C annealing for 1 min, a total of 40 cycles with initials steps of hold at 50 °C for 2 min and 95 °C for 10 min in 96-well optical reaction plates (Applied Biosystems, USA). The target gene expression was normalized with Plant 18S rRNA gene which was used as the endogenous control for the entire study (Table 2).

Results and Discussion

The Data Analysis was performed using Data Assist Software - Applied Biosystems. The Gene expression data is best represented as Heat Map, where green indicates higher expression, pale green indicates lower expression and Red indicates expression beyond scale or over expression. The transcript

Table 2. Primers used for amplifying in Real-Time PCR

SI. No	Product/ Target Name	Primer
Custom Taqman Based Primers		
1	TR19630|c0_g1	Forward: ACAAGGAAGCCCGTGTATTG Reverse: TGGATTGGTCCTTCGCTATC
2	TR26753|c0_g1	Forward: GACACCGCATACGTACATCG Reverse: AGCGTTTTCACCGAAAGAGA
3	TR52087|c0_g4	Forward: TTGTTGAGCAACGAGACCAC Reverse: TCGTTATCATCCAGCAGCAG
4	TR26739|c0_g3	Forward: GGCAAACATATCGGCAAAGT Reverse: CCTCCAAGACCACCGACTAA
5	TR16494|c0_g4	Forward: TGAGTGGCAGCAGCAGTAAC Reverse: CCTCCTGGTAATCTCCGAT
6	TR54668|c0_g1	Forward: TCAGCAATAGAACCCCGAAC Reverse: ACATTTGCTGGGTTCAGGAC
7	TR41913|c0_g1	Forward: CAAAAGAAGCCGACCATCAT Reverse: CCCTGACGAAATGCAAATCT
8	18S rRNA	Forward: ATTCTATGGGTGGTGGTGC Reverse : CCATCCAATCGGTAGGAGC
		SYBR Green Based Primers
1	TR83729|c0_g2	Forward: GCATGAAGAACTCCAGCACA Reverse: CAGCAAGGAAAGGGTGTAGC
2	TR20915|c1_g1	Forward: TAGGGTGCGGAGATTGTAGG Reverse: ACACCCCACACCACACCTAT
3	TR15761|c0_g1	Forward: TCCTGGAAAGCGAAGAGGTA Reverse: CGTCGTCCCGAAAGTTACAT
4	TR38214|c0_g1	Forward: ATGAATGGCAACCTGCTCTC Reverse: TTGATGCCAAAACCAACTCA
5	TR41764|c0_g1	Forward: GCAGTTTGGTGTGGGTTTCT Reverse: GTTCCTCTGCCAAGTTGCTC
6	TR15734|c0_g2	Forward: TGTCGCGCTAATCTTGTTTG Reverse: CTTGCAATTGTTCCCAGGTT

Source: Tripathi *et al.* 2017

TR19630|c0_g1, shows significantly higher expression at 75%, 50% and 25% of induced drought condition while the TR41913|c0_g1 shows significant expression at 50% and 25% induced drought condition. Similarly the TR19630|c0_g1 and TR41913|c0_g1 were significantly under expressed when only 100% and 75% of drought condition was induced to the teak tree leaves (Fig. 1). The TR15761|c0_g1 showed significantly higher expression when the leaves were induced with 75% and 25% drought condition, while when subjected to 100% and 50% drought the transcript expression was significantly low. TR41764|c0_g1 showed significantly higher expression when the leaves were induced with 50% and 25% drought condition, while when subjected to 75% and 100% drought the transcript expression was significantly low (Fig. 2). The transcript TR26753|c0_g1 showed significantly higher expression when the leaves were induced with 50% and 100% drought condition, while TR26753|c0_g1 when subjected to 25% and 75% drought the transcript expression was significantly low (Fig. 3). TR52087|c0_g4 showed significantly higher expression when the leaves were induced with 25% and 75% drought condition, while when subjected to 100% and 50% drought the transcript expression was significantly low. TR16494|c0_g4 showed significantly higher expression when the leaves were induced with 50% and 25% drought condition, while when subjected to 75% and 100% drought the transcript expression was significantly low (Fig. 4). TR54668|c0_g1 showed significantly higher expression only when the leaves were subjected to 100% drought condition, while the expression was significantly under expressed at 75%, 50% and 25% condition. Whereas TR15734|c0_g2 signifi-

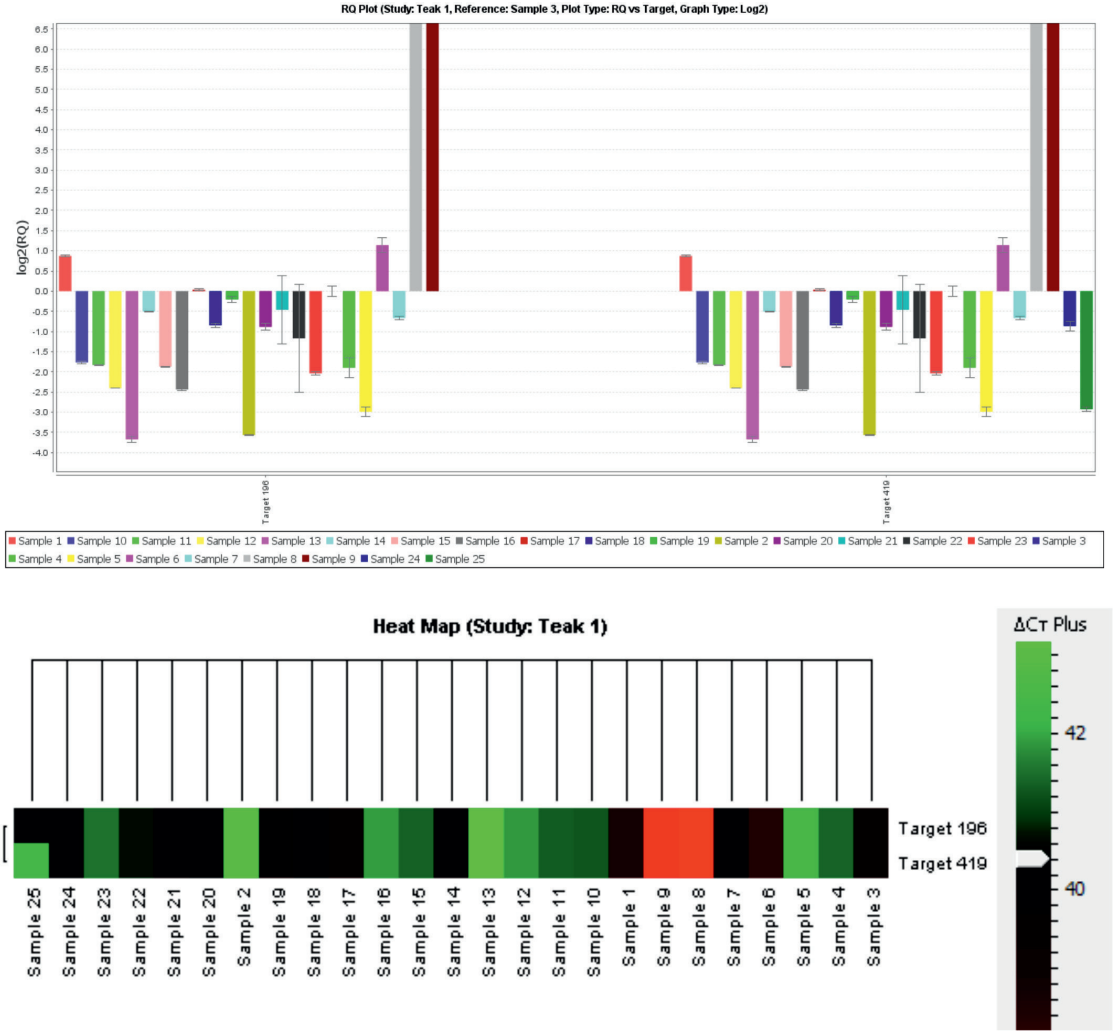

Fig. 1. Heat Map and RQ Plot for **TR19630|c0_g1 and** TR41913|c0_g1 normalized with 18S rRNA

cantly higher expression when the leaves were subjected to 75% and 50%, while lower expression was observed of TR15734|c0_g2 at 25% and 100% drought condition (Fig. 5). Figure 6 shows the expression pattern for transcript TR20915|c1_g1 in Teak tree leaves which were subjected to 100%, 75%, 50% and 25% drought condition. It was observed that the transcript TR20915|c1_g1 showed significantly higher expression when exposed to 25% and 50% drought condition when compared to 75% and 100%. Figure 7 indicates that the TR26739|c0_g3 showed significantly higher expression when the leaves were induced with 100% drought condition. While in other higher or prolonged drought conditions, the transcript expression was found significantly low. Figure 8 indicates that TR83729|c0_g2 showed significantly higher expression when the leaves were induced with 25% drought condition. While in other drought conditions, the transcript expression was found significantly low. Figure 9 indicates that TR38214|c0_g1 showed significantly lower expression when the leaves were induced with all the drought conditions from 100% to 25%.

The Study emphasizes the role of transcripts TR19630|c0_g1, TR26753|c0_g1, TR52087|c0_g4, TR26739|c0_g3, TR16494|c0_g4, TR54668|c0_g1, TR41913|c0_g1, TR83729|c0_g2, TR20915|c1_g1, TR15761|c0_g1, TR38214|c0_g1, TR41764|c0_g1 and TR15734|c0_g2, from Teak tree and how the expression pattern varies when exposed to various drought conditions of 25%, 50%, 75% and 100%. Based on the observations when the tree is subjected

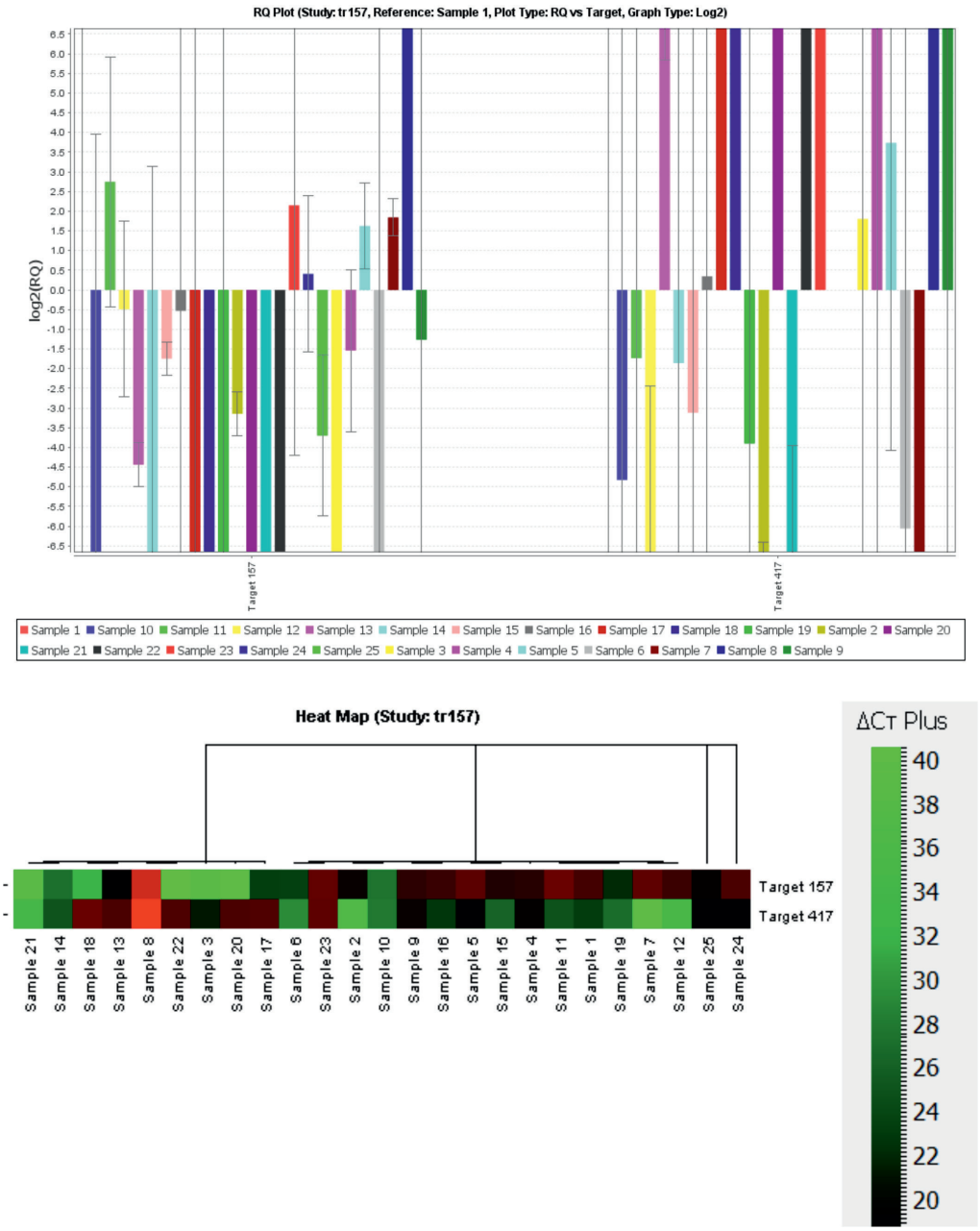

Fig. 2. Heat Map and RQ Plot for TR15761|c0_g1 and **TR41764|c0_g1** normalized with 18S rRNA

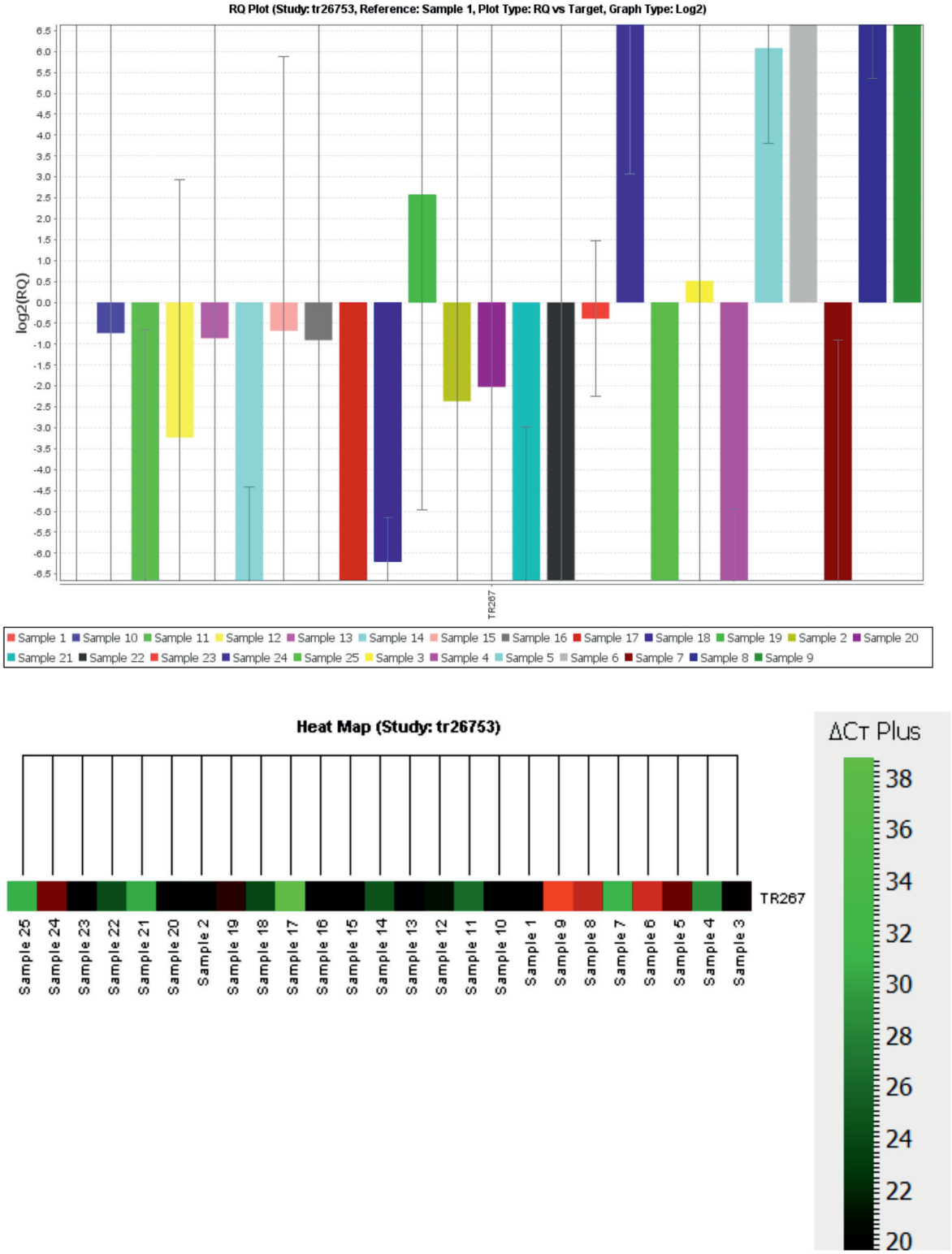

Fig. 3. Heat Map and RQ Plot for TR26753 | c0_g1 normalized with 18S rRNA

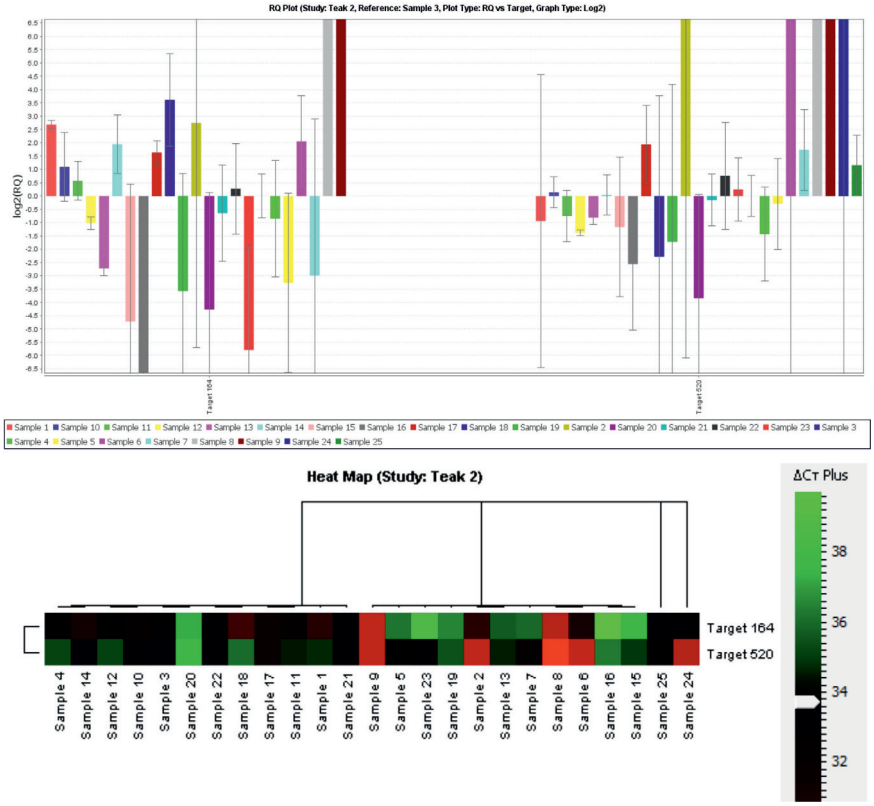

Fig. 4. Heat Map and RQ Plot for TR52087 | c0_g4 and TR16494 | c0_g4 normalized with 18S rRNA

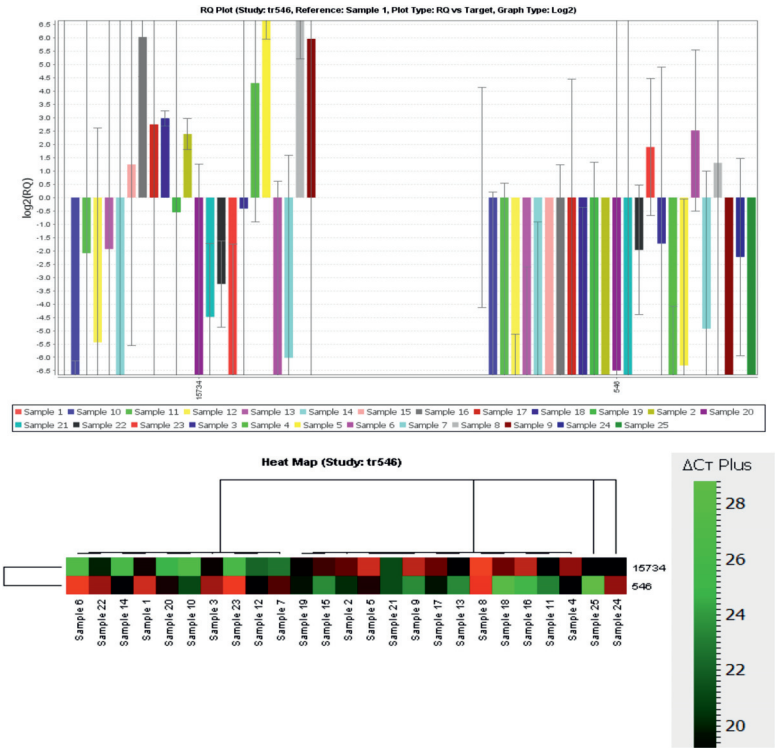

Fig. 5. Heat Map and RQ Plot for TR54668 | c0_g1 and **TR15734 | c0_g2** normalized with 18S rRNA

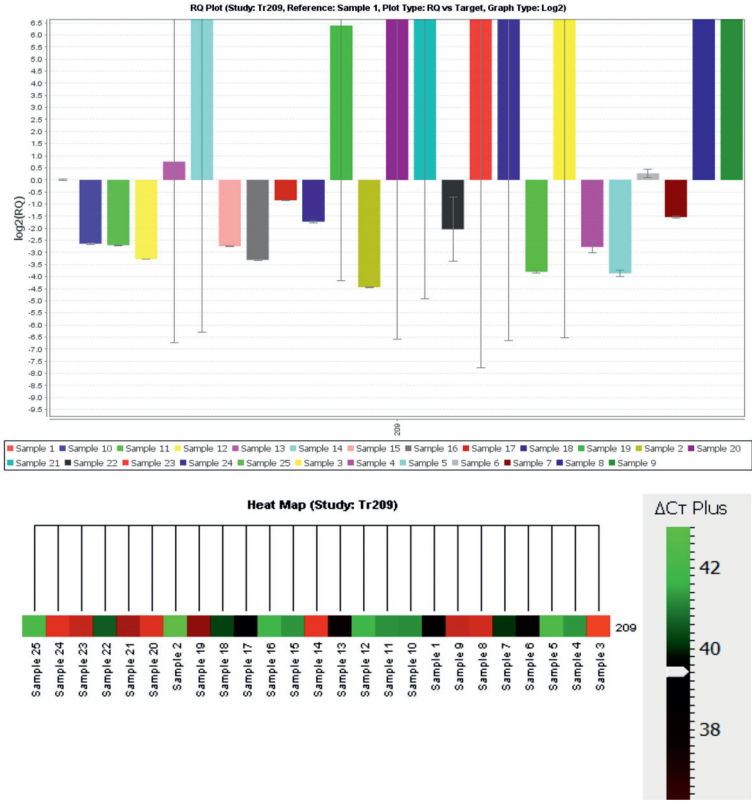

Fig. 6. Heat Map and RQ Plot for TR20915 | c1_g1 normalized with 18S rRNA

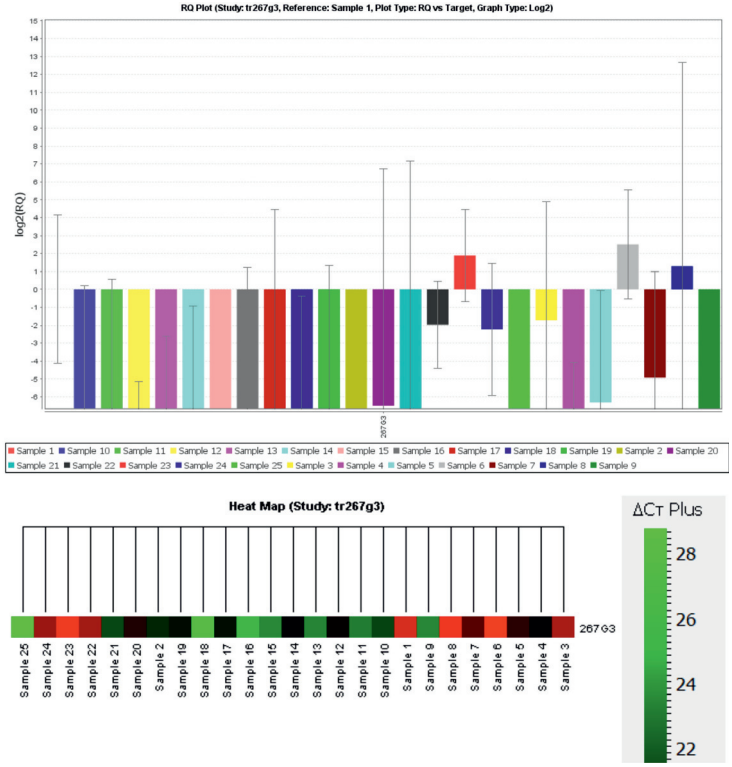

Fig. 7. Heat Map and RQ Plot for TR26739 | c0_g3 normalized with 18S rRNA

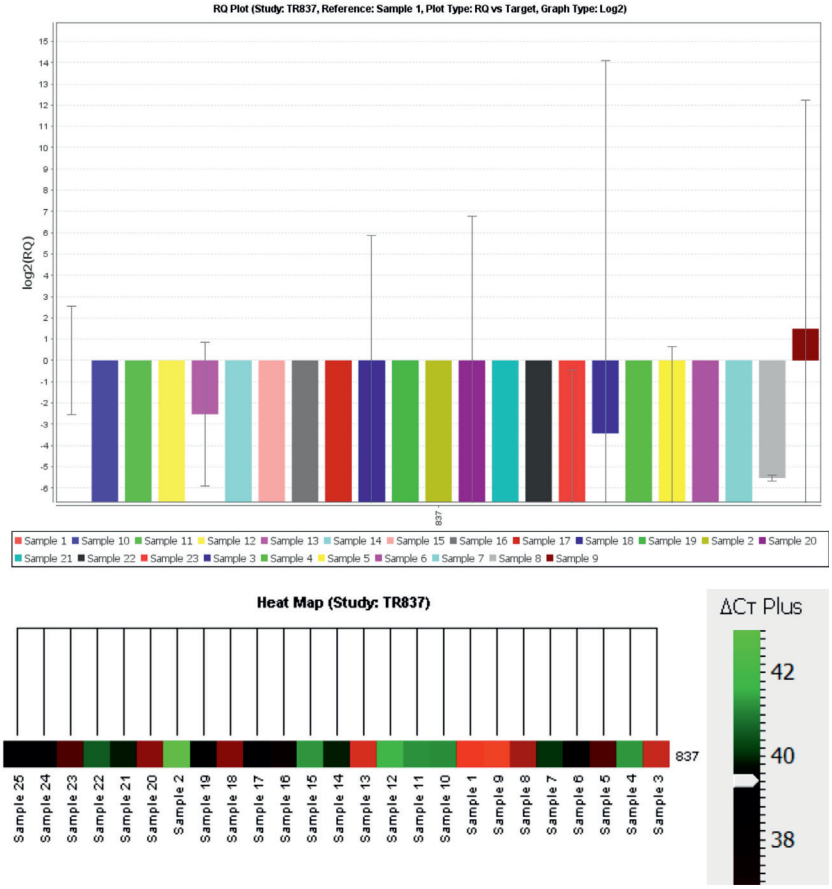

Fig. 8. Heat Map and RQ Plot for TR83729 | c0_g2 normalized with 18S rRNA

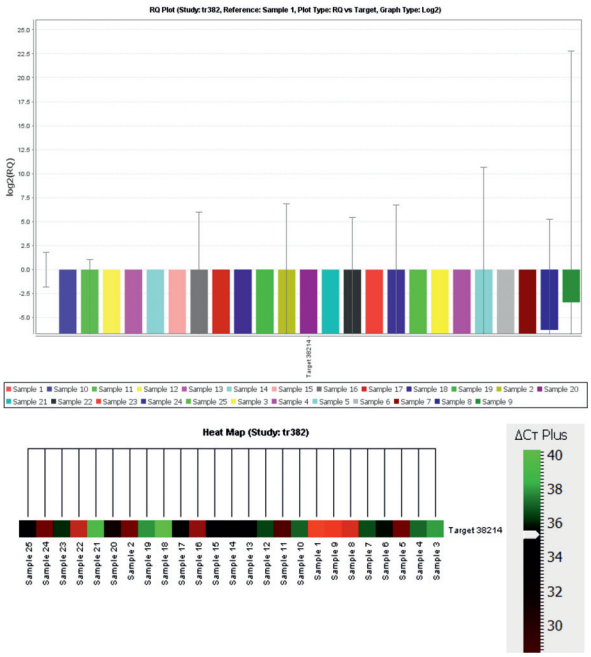

Fig. 9. Heat Map and RQ Plot for TR38214 | c0_g1 normalized with 18S rRNA

to 25% drought condition, the following transcripts have showed increased expression, TR19630 | c0_g1, TR41764 | c0_g1, TR26753 | c0_g1, TR16494 | c0_g4 , TR15734 | c0_g2, and TR20915 | c1_g1. This indicates these transcripts may be responsible or initiate cell apoptosis, leading to *cell* shrinkage, cytoplasmic condensation, chromatin condensation, and DNA fragmentation which finally caused the shrinkage and wilting of leaves, induced by drought conditions. However, it was also observed that the transcript TR38214 | c0_g1 has shown lower expression in all the drought condition, which possibly indicates the transcript induces a protective mechanism which ensure the plant leaf cells do not undergo apoptosis despite harsh environmental conditions. Further bioinformatics bases pathway analysis can clearly elucidate the mechanism by which these transcripts exert their function on teak tree leaves leading to apoptosis.

Conclusion

Previous studies suggest that the plants is a phreato-

phytic species, its drought tolerance strategy is probably more related to its great root growth capacity rather than its ability to tolerate dehydration (Gries *et al*., 2003; Hukin *et al*., 2005). It was also demonstrated that shutting down the plants growth inhibition under moderate water stress is more valuable than engineering plants that can survive in extreme drought conditions as drought is rarely severe enough to kill plants in natural environment but rather reduces plant growth (Marshal *et al*., 2012). Our research may provide new insights into the molecular mechanisms of *T. grandis*'s response to drought stress and help engineering crops that can maintain production under moderate drought stress. From the experiments examining relative expression of genes involved in water stress, the most interesting gene to be studies for teak in future is TR38214|c0_g1, as it has shown low expression in all drought conditions. Further studies of this gene under different biotic and abiotic stress treatments are needed.

Acknowledgement

The authors are grateful to the Director, CSIR-North East Institute of Science & Technology, Jorhat, Assam, India for his permission to publish the work.

Conflict of Interest

The authors declared that they have no competing interests.

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